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TITLE: Modified-fat nutritional products useful preventing or treating obesity

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ABSTRACT:

The present invention provides dietary products for infant child and adult nutrition which possess adequate levels and ratios of medium chain fatty acids and .omega.-polyunsaturated fatty acids. Consumption of these dietary products can contribute to the prevention of obesity in developing individuals and can contribute to a reduction in body fat mass in individuals who are trying to loose weight or reduce body fat mass (e.g., obese individuals). A first preferred product is a dairy supplement or formulated dairy product for consumption by infants or children to prevent development of obesity. A second preferred product is a dietary suppplement for persons combating unwanted weight gain or obesity. Also featured are methods of formulating these dietary products.

Summary of Invention Paragraph - BSTX (6):

[0006] The present invention features dietary supplements and products aimed at preventing obesity, reducing fat mass, and/or reducing serum TGs (in particular, serum TGs associated with traditional MCT diets). In one embodiment, the invention features a

milkfat-derived MCT-rich component that contains an appropriate ratio of milkfat-derived MCFAs to milkfat-derived LCFAs (e.g., between 5:1 to 10:1) and a sufficient amount of -3 PUFAs (e.g., between 1% and 5%). In another embodiment, the invention features a dairy product for human consumption comprising the milkfat-derived MCT-rich component of the present invention, preferably a milk for human consumption comprising the milkfat-derived MCT-rich component of the present invention. In yet another embodiment, the invention features a dietary supplement that includes an appropriate ratio of MCFAs to LCFAs, a sufficient amount of -3 PUFAs and a protein source (e.g., a soy protein source). In a preferred embodiment, the dietary supplement does not include a carbohydrate source.

Brief Description of Drawings Paragraph - DRTX (2):

[0007] FIG. 1 .sup.13C-NMR spectra of lipid extracts after cells were incubated with [1-.sup.13C]fatty acids. (A) Oleate and (B) octanoate in 3T3-L1 fat cells, and (C) octanoate in HepG2 cells. Above the spectra is shown a molecular formula of palmitoleate, a common end product of de novo synthesis of LCFA. The spectrum was obtained with 2000 (A) and 4000 (B and C) scans.

Brief Description of Drawings Paragraph - DRTX (6):

[0011] FIG. 5 Graphic depiction of the effects of octanoate on oleate storage and glucose conversion to the glycerol backbone in TG. (A) Incorporation of [I-.sup.14C]oleate into cellular TG as a function of increasing octanoate concentration; (B) [1-.sup.14C]glucose conversion to the glycerol backbone in TG in fat cells treated with octanoate, oleate or octanoate plus oleate.

Detail Description Paragraph - DETX (2):

[0025] The present inventors have discovered that MCFA can regulate both triglyceride storage and differentiation of fat cells. In particular, octanoate is more oxidized than stored by fat cells, in contrast to oleate which is more stored. The accumulation of oleate into fat cell TGs increases with both time and concentration of exogenously added oleate, whereas octanoate incorporation becomes saturated and accounts for only about 10% of total fatty acids stored. Fat cells pretreated with octanoate had a significantly enhanced rate of TG hydrolysis. Moreover, octanoate but not oleate, prevents the differentaition of fat cells.

Detail Description Paragraph - DETX (3):

[0026] Understanding the mechanisms by which MCFA regulate metabolism of fat cells has allowed the present inventors to formulate dietary supplements and products aimed at reducing fat mass

during development. This reduced fat mass will result in subjects being less sensitive to diet induced obesity later in life.

Detail Description Paragraph - DETX (28):

[0051] The phrase "adequate" ratio, e.g., an adequate ratio of MCFAs to LCFAs, is a ratio that facilitates reduced triglyceride storage in peripheral cells (in particular, fat cells) and/or reduced adipogenic differentiation and/or reduced fat mass in a subject. The phrase "sufficient" amount of PUFAs, e.g., a sufficient amount of -3 PUFAs, is an amount that detectably reduced serum triglycerides as compared, for example, to serum triglyceride levels in a subject supplementing their diet with MCTs or MCFAs or consuming products having high MCFA:LCFA ratios.

Detail Description Paragraph - DETX (44):

[0066] The following Examples demonstrate, in addition to being directly metabolized by liver cells, have metabolic effects on peripheral cells, in particular, fat cells. The Examples demonstrate that MCFA are capable of effecting TG storage, TG esterification, lipolysis, and fat cell differentiation. Moreover, controlling the ratio of MCFAs to LCFAS and incorporating a sufficient amount of PUFAs into preferred fat mixtures can effect not only fat storage but levels of circulating TGs as well. Based on these heretofor unrecognized metabolic effects of MCFA, dietary formulations are taught that can be used to supplement the diets of persons having normal fat stores or excess fat stores, to prevent fat cell differentiation and/or lipid accumulation.

Detail Description Paragraph - DETX (47):

[0069] Medium-chain triacylglycerols (MCT) have been used as nutrients for patients with disorders of long-chain triacylglycerol (LCT) or glucose metabolism for decades. Several early studies demonstrated that MCT diets prevented weight gain in animals [8-10] without affecting plasma cholesterol or other physiological parameters [11,12]. Feeding MCT early in life influenced adipose-tissue development and resulted in fewer and smaller fat cells with less lipid [10]. Neurotoxicity [13] and ketosis [14] have only been reported after acute, high-dose MCT intravenous infusion in animals. Recent trials have demonstrated that the addition of MCT to human diets is of benefit for certain dyslipidaemic disorders including diabetes [15,16]. The rationale for these therapeutic benefits is not fully understood. The potential applications of MCT in the treatment of obesity have been reviewed in [17,18].

Detail Description Paragraph - DETX (61):

Both Octanoate and Oleate are Actively Metabolized by Fat Cells, However, Octanoate is More Oxidized than Stored

Detail Description Paragraph - DETX (66):

Both Octanoate and Oleate are Direct Incorporated by Fat Cells into Cellular Lipids

Detail Description Paragraph - DETX (68):

[0086] [1-.sup.13C]oleate and [1-.sup.13C]octanoate were each found to be esterified to TG at the sn-1,3 as well as the sn-2 positions in fat cells (FIG. 1), represented by the peaks arising from the corresponding carbonyl resonances, TG(1,3) and TG(2). Such direct esterification of [1-.sup.13C]octanoate was not detected in HepG2 cells (FIG. 1C), whereas [1-.sup.13C]oleate was directly esterified in HepG2 cells [30] to an extent similar to that in fat cells (results not shown). The amount of [1-.sup.13C]oleate or [1-.sup.13C]octanoate incorporated into phospholipids was insignificant, as evidenced by the lack of corresponding resonances [25].

Detail Description Paragraph - DETX (71):

[0087] In principle, the acetyl-CoA derived from the .beta.-oxidation of [1-.sup.13C]FFA can be used for de novo FFA synthesis. Any incorporation of [1-.sup.13C]acetyl-CoA into the acyl methylene would be detected by NMR. In previous studies on fat cells treated with oleate or palmitate, partitioning of exogenous fatty acids into this pathway was not detected [25,27,31]. However, for cells incubated with octanoate, it was found that the integrated intensities of some methylene peaks representing a single carbon (.alpha.+1, .alpha.-1, etc.) were about 2-fold more intense than the .omega.CH.sub.3 peak (FIGS. 1B and 1C), indicating selective labelling of the aliphatic region with .sup.13C isotope. Peaks for the .alpha.CH.sub.2 and (.alpha.+1)CH.sub.2 generally were broader or split because of the magnetic shielding from sn-1,3 or sn-2 carbonyls. Therefore, the peak heights of these signals were lower than the signals arising from the other methylenes even though they may have had the same overall integral intensity.

Detail Description Paragraph - DETX (74):

MCFA are Stored Less Than LCFA in Fat Cells

Detail Description Paragraph - DETX (75):

[0089] It is well accepted that MCFA are readily oxidized with minimal esterification into triglycerides in fat cells. Using a new NMR procedure, it could be demonstrated that octanoate was stored

less than oleate, but still to a significant extent, implying that MCFA may have more metabolic influence on fat cells than simply a quick energy substrate. FIG. 2 compares the esterification rate of MCFA (C8, C10, C12) to that of LCFA (C18:1) in cultured adipocytes. The data clearly show that cells exposed to MCFA accumulated less lipid compared to those exposed to LCFA of equal concentration.

Detail Description Paragraph - DETX (84):

[0094] When added to undifferentiated cells (not treated with MDI), lipid droplets began to appear in cells treated with oleate 3 days after the incubation. In octanoate-treated and control cells lipid droplets began to appear 6 days after incubation, but to a much lesser extent than in cells treated with oleate. After 9 days of incubation, about 90% of the cells contained lipid droplets. The droplets in octanoate-treated cells (FIG. 4A) were much smaller than those in oleate-treated cells (FIG. 4B). After extended fatty acid incubation, there was about a 20% cell loss in oleate-treated cultures, as shown by microscopic examination (FIG. 4) and corroborated by DNA analysis. Such cell loss was less significant in octanoate-treated cells. Since the cells that lifted off were mostly differentiated fat cells (examined by microscopy), the cell loss was likely to be induced by the propensity of fat-laden cells to float rather than by fatty acid-related toxicity, although the rapid lipid accumulation in the presence of excess oleate may have accelerated this process. When cells were differentiated with MDI treatment and subsequently accumulated lipids mostly by de novo synthesis from glucose, cell loss was not significant up to 6 days after MDI treatment.

Detail Description Paragraph - DETX (86):

[0096] It was then determined whether the observed difference in cellular lipid storage was simply because less MCFA was esterified than LCFA or whether MCFA also affected the storage of LCFA. When [1-.sup.14C] oleate was added to the culture medium in the presence of unlabeled octanoate, the incorporation of .sup.14C isotope into cellular lipids was decreased in proportion to the exogenous octanoate concentration (FIG. 5A). Since glycerol kinase activity is minimal in fat cells, glucose is the major supplier of the glycerol backbone for triglyceride synthesis. Therefore, the amount of [1-.sup.14C]glucose-derived isotope incorporated into the TG-glycerol backbone reflects the net TG synthesis in the cell. As shown in FIG. 5B, cells exposed to octanoate had a lower TG synthesis rate than cells exposed to oleate, and the replacement of oleate partially by octanoate reduced the net TG synthesis in adipocytes. These data demonstrate that MCFA down regulate lipogenesis and reduce adipocyte fat content.

Detail Description Paragraph - DETX (90):

MCFA Inhibit Preadipocyte Differentiation into Fat Cells and Down Regulate Adipogenic Gene Expression in Mature Adipocytes

Detail Description Paragraph - DETX (112):

[0113] Fatty acids of different chain lengths have different effects on cellular processes. Whereas the pathological roles of saturated compared with unsaturated LCFA are well established [36], the effects of MCFA are far less understood. The data presented in Examples 1-10 address issues including how much octanoate is stored by fat cells, how it perturbs the molecular structure of TG, and how it affects cell differentiation. The data demonstrate major differences between octanoate and oleate in their oxidation, esterification and release from TG and their influence on adipocyte differentiation. It has been widely accepted that MCFA are mainly oxidized in cells through the carnitine-independent pathway whereas LCFA may be stored or oxidized depending on the economy of other fuels [37]. This argument has been used to explain the low storage rate of MCFA in fat cells [17]. However, there is evidence that MCFA can be esterified in TG in the liver [38] and fat cells [21,39], which is confirmed by the above-presented in vitro data.

Detail Description Paragraph - DETX (113):

[0114] First, it is demonstrated that octanoate is stored in differentiated fat cells but not in undifferentiated preadipocytes (FIG. 9), although oleate can also be stored in undifferentiated fat-cell precursors [27]. Storage of octanoate increases as cells became more differentiated until a maximum level is reached. It is well known that MCFA have a low affinity for cytosolic acyl-CoA synthase [40], but can be readily activated within the mitochondrial matrix for oxidation. Both factors might lead to relatively low cytosolic substrate availability for esterification. However, these may not be the sole reasons for the low rate of MCFA storage, because increasing the substrate concentration of octanoate (up to 5-fold) and extending the incubation period does not increase the proportion of octanoate in stored fat. Another related factor may be the pool of carnitine, which is needed to transport octanoyl-CoA from the mitochondria to the cytosol, and the pool of CoA, which increases with differentiation (unpublished work) and could lead to an increase of octanoyl-CoA concentration in the cytosol.

Detail Description Paragraph - DETX (115):

[0116] The observation that octanoate incorporation in fat cells becomes saturated and accounts for about 10% of total fatty acids is consistent with previous studies in vivo. [21]. When converted to a molar scale, this accounts for 20% of the total acyl chains stored in fat cells, an amount that would be predicted to have substantial

effects on cellular metabolism. In contrast to the above demonstration that octanoate turnover is much faster than that of oleate (FIG. 11), storage of MCFA in infant subcutaneous fat was previously shown to be rather stable and remained unchanged 1 week after switching to a MCT-free diet [21]. This is likely because subcutaneous-fat turnover rate is intrinsically lower than that in visceral fat since it serves mainly as thermal insulation and mechanical cushioning [42]. Furthermore, visceral fat is affected more by dietary modulation than peripheral fat.

Detail Description Paragraph - DETX (116):

[0117] Third, a significant finding resulting from the above-described stuies is that fat cells pre-treated with octanoate have a significantly enhanced TG hydrolysis (FIG. 10). This finding has not been reported before. It may be argued that less TG storage in cells pre-treated with octanoate results in smaller fat droplets and thus a larger surface area of lipids. However, the cells we used were well differentiated before they were treated with octanoate, and there were no microscopic differences in cell morphology or fat droplet size examined by phase contrast microscopy. The difference in total stored TG was rather small between cells pre-treated with octanoate or oleate, and could not account for the difference in glycerol release (FIG. 10). Instead, the results suggested that incorporation of octanoate may facilitate TG hydrolysis. Since the hydrolysis product MCFA diffuses away from the site of reaction more rapidly than LCFA, the lipase efficiency is higher for MCT than LCT [43]. Furthermore, hydrolysis at the sn-1,3 position is the rate-limiting step in TG lipolysis [44]. Hence, the findings of the preferential incorporation of octanoate at the sn-1,3 position and the increased glycerol release in octanoate pre-treated fat cells fit together logically.

Detail Description Paragraph - DETX (117):

[0118] Yet another significant finding is that the MCFA, octanoate, can actually decrease the amount of LCFA (e.g., oleate) stored as TGs in fat cells.

Detail Description Paragraph - DETX (118):

[0119] Finally, another important observation is that octanoate, in contrast to oleate, does not stimulate fat cell differentiation, even after an extended incubation period. The eventual lipid accumulation is similar to that seen in control cells as a result of limited spontaneous differentiation in the presence of insulin and glucose [34]. In contrast, incubation with oleate rapidly induces differentiation with characteristic marked TG accumulation and increased G3PD activity. This is consistent with previous reports

that LCFA induce the expression of genes involved in fatty acid metabolism [45-47]. This may explain the observation that weaning rats on MCT diets have lower fat-cell numbers as adults compared with their littermates on LCT diets [10]. Furthermore, long-chain CoA esters, but not short- or medium-chain CoA esters, are potent modulators of metabolic enzymes and signal transduction [48,49]. The fact that LCFA induces preadipocyte differentiation in vitro may also correlate with the observations that animals and humans on high-fat diets usually acquire more fat cells than controls [50-52]. Moreover, octanoate can inhibit differentaition of preadipocytes cultured in the presence of hormonal inducers of differentiation. The above-described findings support the hypothesis that replacement of part of the LCFA in conventional high-fat diets with MCFA at critical times in development can serve as a means to control cell number and decrease lipid accretion.

Detail Description Paragraph - DETX (124):

[0121] One of the major concerns of applying MCT in human trials has been that it usually raise the percentage of C16:0 and C18:1, the common products of de novo fatty acid synthesis. It has been reported that MCFA inhibits the acetyl-CoA carboxylase gene cultured chicken hepatocytes (Hillgartner, 1997). To address the possible adverse effects of the MCT diet on plasma lipid profiles by increasing de novo synthesis of LCFA, fish oil was used as a supplement to MCT in the dietary treatment experiments (FIG. 12). Because the .omega.-3 long chain fatty acids (EPA and DHA) have been shown to efficiently inhibit fatty acid synthesis, it is proposed that mixing MCFA with a small portion of EPA or DHA will synergize the positive effects of both types of fatty acids in reducing fat storage in adipose tissue and fat production in the liver.

Detail Description Paragraph - DETX (134):

[0127] Additionally, we studied effects of octanoate on the plasma membrane fatty acid composition in 3T3L1 fat cells incubated with oleate/linoleate by replacing 20% (wt %) of the oleate with octanoate. This reduced the cellular TG storage from 41.5 to 27.0 .mu.g/.mu.gDNA. The fatty acid composition in the plasma membrane phospholipids (FIG. 15) was less drastically affected by exogenous fatty acids than that in the TG fraction (FIG. 14). However, replacement of 20% oleate by octanoate still resulted in .about.20% increase in PUFA, .about.10% increase in SFA, and .about.15% decrease in MUFA in the plasma membrane phospholipids (FIG. 15, right panel).

Detail Description Paragraph - DETX (147):

[0138] 4 Maragoudakis, M. E., Kalinsky, H. J. and Lennane, J. (1975) Metabolism of octanoate and its effects on glucose and

palmitate utilization by isolated fat cells. Proc. Soc. Exp. Biol. Med. 148, 606-610

Detail Description Paragraph - DETX (178):

[0169] 35 Shillabeer, G., Forden, J. M. and Lau, D. C. (1989) Induction of preadipocyte differentiation by mature fat cells in the rat. J. Clin. Invest. 84, 381-387

Detail Description Table CWU - DETL (5):

5TABLE 5 Fatty acid composition of cellular TG before and after 96 h of incubation with DMEM (1% BSA) in cells pre-treated with oleate and octanoate Oleate Octanoate Acyl chains Before After Before After 8:0 0 0 10.33 .+-. 0.3 0.99 .+-. 0.05 14:0 2.84 .+-. 0.09 3.28 .+-. 0.02 3.74 .+-. 0.04 3.88 .+-. 0.21 14:1 4.75 .+-. 0.08 5.48 .+-. 0.03 5.56 .+-. 0.29 5.78 .+-. 0.11 15:0 1.02 .+-. 0.04 1.36 .+-. 0.02 1.43 .+-. 0.14 1.63 .+-. 0.17 15:1 0.81 .+-. 0.01 1.24 .+-. 0.09 1.4 .+-. 0.14 1.41 .+-. 0.09 16:0 20.01 .+-. 0.36 23.65 .+-. 0.03 24.62 .+-. 0.35 24.45 .+-. 0.03 16:1 27.34 .+-. 0.29 38.65 .+-. 0.2 38.61 .+-. 0.2 47.64 .+-. 0.34 17:0 0.82 .+-. 0.03 0.75 .+-. 0.03 0.9 .+-. 0.03 0.85 .+-. 0.096 17:1 3.31 .+-. 0.26 3.98 .+-. 0.07 3.89 .+-. 0.1 4.73 .+-. 0.12 18:0 0.38 .+-. 0.04 0.41 .+-. 0.02 0.49 .+-. 0.02 0.51 .+-. 0.16 18:1 n9 36.4 .+-. 0.4 19.71 .+-. 0.02 6.69 .+-. 0.09 6.2 .+-. 0.29 18:1 n11 1.35 .+-. 0.07 1.51 .+-. 0.12 2.34 .+-. 0.05 1.95 .+-. 0.13 *Results are shown as percentages (means .+-. S.E.M., n = 3). The appearance of odd-number chain-length fatty acids is typical in 3T3-L1 fat cells as a result of de novo synthesis [50]. Other fatty acids, including C18:2 and C18:3, were also detected, but to a lower extent.